ID China bio-gas experimentations

法国发起发展组织昆明办公室"沼气池优化管理"
及"沼渣沼液多用途"系列研究

USING BIO-SLURRY FOR PEST CONTROL
沼渣沼液作为杀虫剂的功效研究

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1 ABSTRACT 摘要

As part of its commitment to provide continuous support to the more than 2500 families using a biogas digester build by Initiative Développement (ID), the NGO has engaged in a one year study focused on optimising the management of biogas digesters, as well as researching the various uses of the bio-slurry.

In particular, ID has researched how the bio-slurry could be used by the households as a pesticide. Three families were recruited amongst ID's biogas beneficiaries and made a plot available for field trials. Two experiments were made on **apple tree** in Lianhe 联合village, Sayu 洒渔 township, while experiment was made on **tobacco** in Zhongying 中营 village，Xiaolongdong 小龙洞 township，both in Zhaotong 昭通 prefecture, Yunnan 云南 province, China. The households provided bio-slurry from their own tanks as well as their usual synthetic pesticides for comparison. The experiments were held between March and August 2013.

In the experiment of bio-slurry on apple trees, each orchard was divided into three treatments. In Group 1, the apple trees were sprayed with bio-slurry only; in Group 2, both pesticides and bio-slurry were sprayed; in Group 3, the apple trees were sprayed with pesticides only, according to the beneficiaries' usual practice.

In the experiment of bio-slurry on tobacco, the field was divided in two treatments. In Group 1, only bio-slurry was sprayed on the leaves as well as used on the soil; while in Group 2, only pesticides were sprayed.

In each group, counts of pests and diseases were done every three weeks. On tobacco, significant reductions in the diseases were observed where bio-slurry was sprayed (significance level 0.05): incidence of tobacco mosaic was reduced by 58-75%, brown spot was reduced by 47-70% and tobacco weather fleck was reduced by 81%. The lushness of the leaves was also increased. The differences between the groups were visually obvious and convincing to the farmer. However, bio-slurry had negative side-effects on curing, probably because its foliar and soil application provided excessive nitrogen to the leaves. The exact mechanism of action of the bio-slurry is not known, whether direct action on the diseases, indirect through higher fertilisation, suppression of the disease vectors, or others.

On apple tree, results were mixed and uncertain. Neither the beneficiaries nor the staff of ID China did observe obvious results. A large majority of the quantitative pest/disease counts were non-significant at significance level 0.05. The experiment suffered from very heterogeneous conditions in the orchards, and its sample ought to have been larger. Undoubtedly however, it has been observed that bio-slurry did not provide a radical control of the pests.

法国发起发展组织Initiative Développement (以下简称ID)为2500余户农村家庭建设了户用沼气池，为了向受益者提供持续的项目后期维护，ID进行了为期一年的“沼气池优化管理”和“沼渣沼液多种用途”研究。

ID尤其针对沼液作为杀虫剂的功效进行了研究。我们在ID户用沼气池项目实施地云南省昭通市，从受益者中选择了三家自愿协助试验的农户，并确定了试验地块：1. 在洒渔乡联合村的苹果树果园里进行了两个试验；2. 在小龙洞乡中营村的一块烟叶地里进行了试验。农户为试验提供了自家沼气池里的沼液，以及他们经常使用的化学杀虫剂。试验时间为2013年3月至8月。

苹果树果园被分为三组：第一组仅施用沼液；第二组同时施用化学杀虫剂及沼液；第三组仅施用化学杀虫剂，这也是当地村民的普遍做法。

烟叶田被分为两组：第一组利用沼液进行叶面及土壤施肥；第二组施用化学杀虫剂。

每三周我们对每个实验组的病虫害进行计量。在施用了沼液的烟叶田里，病害明显减少，病害差异显著性程度达到0.05, 烟草花叶病的发病率降低58-75%，褐斑病发病率降低47%-70%，气候斑点病发病率降低81%, 烟叶也更加茂盛。各实验组植株的外观差异明显，对村民来说也十分具有说服力。然而，沼液对植株的生长产生了负面影响，可能是因为用其进行叶面及土壤施肥给植株提供了过量的氮。沼液的作用机制目前尚不清楚，是否是直接作用于病害，或是其提高了所施肥料的浓度而间接抑制了病害，又或是其他原因。

对苹果树进行的试验，结果复杂而不确定。无论是协助试验的村民还是ID工作人员都没有观察到沼液作为杀虫剂对果树产生明显效果。差异显著性程度0.05的说明果树病虫害发生数量的计算结果没有显著意义。试验受到了果园复杂因素的影响，其样本应该更多。对试验结果进行观察表明沼液对虫害的控制没有起到积极影响。

2 RATIONALE

"Bio-slurry" is the residue of the fermentation of biomass in a biogas digester. The bio-slurry is rich in various mineral and organic elements that render it useful for a variety of agricultural uses including plant fertilisation, as a natural pesticide, or for pre-soaking seeds. These uses are widely promoted by development organisations and governmental agencies throughout the world. In China in particular, the government publishes training material on how to use the bio-slurry.

Illustration 1: Bio-slurry is experimented as a foliar spray, Zhaotong, 2013

As part of its continued support, ID China has been giving regular training courses and advices to the beneficiaries of its biogas programs. Amongst others, the proper use of bio-slurry is taught. However, most of the knowledge and material used by ID China is coming from second-hand sources. Hence, ID China has launched a project of terrain experimentation to gain a direct and in-depth understanding of the use of bio-slurry for agriculture.

The principle behind this project is to involve ID China in partnership with beneficiaries into real-life, terrain-oriented experimentations. The beneficiary provides a field for the trials, which he manages according to a protocol agreed between ID China and himself, while ID China is responsible for the regular qualitative and quantitative monitoring. The goal of the experiment is not only to confirm the validity of the scientific knowledge on the terrain, but also to bring the beneficiary to judge the results by himself. If positive and tangible results are achieved, the beneficiary is motivated to adopt the new and beneficial technique, and become a hinge for the spread of the new knowledge within the local community.

Furthermore, once the potential uses of bio-slurry have been highlighted, what would otherwise only have been a by-product of biogas production can acquire a new/higher value in the eyes of the beneficiaries. This value acts as a secondary motivation, or co-advantage, in the use of the bio-digester. Developing the use of bio-slurry can therefore contribute to the long-term success of a rural biogas program.

Specifically, the experiments aimed at the following goals:

- In general, on apple trees and tobacco, evaluate the feasibility of using bio-slurry in addition, or in substitution of pesticides.
- Compare the efficiency of three different treatments: 1) only bio-slurry; 2) the combination of both pesticides and bio-slurry; 3) only pesticides.
- Research potential adverse effects of bio-slurry, such as damage on the crops, and estimate a safe concentration and application frequency

Illustration 2: Apple orchard in Zhaotong, Yunnan, China

3 METHOD

3.1 Experiment planning and preparation

3.1.1 Recruiting farmers and selecting the plots

In the summer of 2012, about 35 surveys were carried out amongst the beneficiaries of ID China's biogas programs to learn about the households' agricultural practises and their use of the bio-slurry. According to these surveys, it was established that the main crops grown locally, on which bio-slurry could be used for pest control, were corn, potato, tobacco, apple trees, cherry trees and radish. It was decided to carry out pest control experimentations on apple trees and tobacco.

Beneficiaries were recruited amongst the 2500 biogas households which built a digester with the help of ID China since 2008 in Zhaotong 昭通, Yunnan province, China. The criteria for selecting the households included being able to provide a small field for the trials, and having a bio-digester currently in use. Furthermore, a genuine motivation, sense of responsibility and reliability were sought from the households participating in the experiment. The households were not remunerated, although a few small gifts were brought, such as biogas spare parts or fresh fruits/vegetables. An insurance mechanism was agreed in case of loss of harvest (more details below).

According to these criteria, three households were recruited in autumn 2012 (table 1). The orchards and fields on which to carry out the experiments were selected for their homogeneity (soil, slope, sun, tree varieties) while avoiding proximity with other similar crops to reduce the chances of cross-contamination of pests and diseases. Oblong field shapes were preferred to reduce the interaction between the different experimental groups.

Name of responsible person	Township	Village	Crop(s)	Surface of plot 亩 (mu)	Number of trees
Ben 1	洒渔 (Sayu)	联合 (Lianhe)	Apple (Fuji)	1.4	50
Ben 2	洒渔 (Sayu)	联合 (Lianhe)	Apple (Fuji)	1.4	77
Ben 3	小龙洞 (Xiaolongdong)	中营 (Zhongying)	Tobacco	0.8	

Table 1: List of households participating in the experiments

3.1.2 Experimental groups

3.1.2.1 Apple

Three different treatments were tested:

- **Group 1:** spraying of bio-slurry on the leaves, no use of synthetic pesticides
- **Group 2:** spraying of a mixture of bio-slurry and synthetic pesticides on the leaves
- **Group 3:** spraying of synthetic pesticides on the leaves (no bio-slurry)

No control group (using no treatment at all) was implemented, because it was feared that suppressing all pest control could lead to damages to the crop. Furthermore, the small size of the orchards did not allow for reasonably delimiting 4 groups; the 3-group option above was preferred.

The bio-slurry used on groups 1 and 2 was diluted with water to avoid damaging the leaves by excessive nutrient concentrations. Group 2 used the combination of the treatments from groups 1 and 3. Typical mixing proportions for one sprayer (20l) are illustrated in table 2.

The total amounts sprayed per tree, or per unit of field surface, were left to the farmer's experience.

	Group 1	Group 2	Group 3
Water	10l	10l	20l
Bio-slurry	10l	10l	
Pesticides		According to beneficiary	According to beneficiary (same quantity per sprayer as in group 2)

Table 2: Typical proportions for spraying on groups 1,2,3 (apple)

3.1.2.2 Tobacco

On tobacco, only two treatments were tested. No control group was established.

- **Group 1:** Pest control first through synthetic pesticides, then replaced with bio-slurry in foliar spray. Additionally, application of bio-slurry on the soil.
- **Group 2:** Pest control through synthetic pesticides (no use of bio-slurry on leaves or soil)

3.1.3 Role of the beneficiaries

The work and management on the apple orchards and the tobacco field were entirely left to the beneficiaries. This included the spraying of bio-slurry and/or pesticide, pruning, fertilisation, and harvest. The beneficiaries were responsible for choosing themselves the pesticides, their quantity and application methods. For the bio-slurry (which none of the farmers had used before) ID China provided some advice beforehand, thereafter the beneficiaries were left to experiment the proper application methods and rates. However, every spraying operation, as well as every fertilisation, had to be written down in a "logbook" (illustration 3) provided by ID China, indicating the date, the product used, and the quantity.

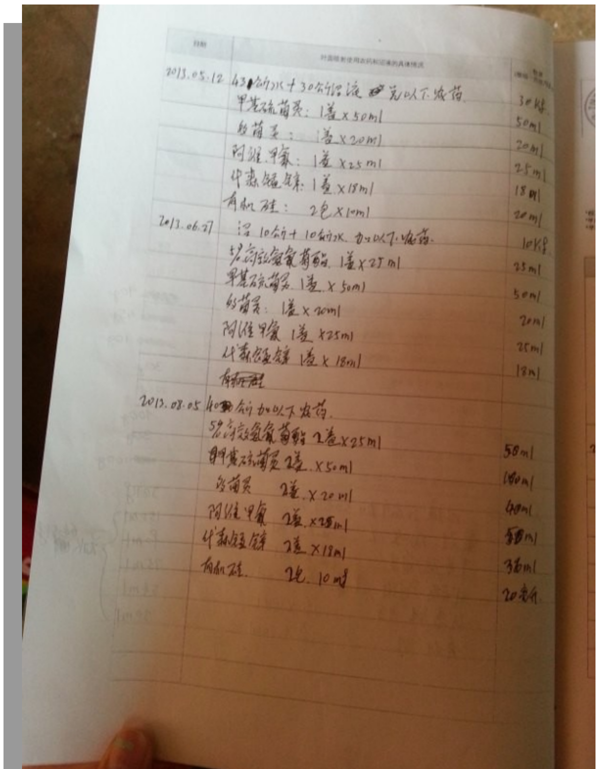


Illustration 3: "Logbook" used by the beneficiary to keep track of spraying

The beneficiaries were in first line for observing and evaluating the efficiency of the treatments and reporting any observed differences or interesting facts. Since the team of ID China could only visit the terrain every two to three weeks, the qualitative judgement/monitoring relied therefore essentially on the beneficiaries. We met the beneficiaries on nearly every field visit and listened to their remarks and observations. To complement the farmer's opinion with an objective method for judging the efficiency of the bio-slurry, the team of ID China carried out quantitative insect screenings group per group.

3.1.4 Guarantee for the harvest

As a side effect of the experiment, the health or yields of the crops could have been affected on the groups on which bio-slurry was used, and especially on the group were pesticides would be suppressed. ID China therefore proposed an insurance mechanism to compensate financially any potential losses attributed to the experiment. Surprisingly, all three beneficiaries were displaying very strong confidence in the experiment and initially brushed aside the proposal on the motive that the harvest was not at stake. However, to prevent any possible future misunderstanding, ID China insisted on passing an insurance scheme and a contract was signed. The deal proposed a compensation in cash equal to the loss of harvest, if any, at the current market price (yields were to be weighed at the end of the experiment, if necessary).

3.1.5 Delimiting the plots

The experiments were designed to closely follow real terrain conditions amongst the beneficiaries of ID China, therefore the chosen fields/orchards were small and irregularly shaped, representative of the fragmented and hilly agricultural landscape of northern Yunnan. One orchard was even split in two terraces on the slope. Larger fields could not have been found amongst the beneficiaries of ID China, which are, by reason of the NGO's mission, chosen amongst the less well-off farmers. Such conditions, however, worked against experimental accuracy, which would have required larger, regular, well demarcated plots.

Three groups were delimited within each orchard and two on the tobacco field. Concerns of cross-contamination dictated, as much as possible, to minimise the interactions between the different experimental groups. For this reason, the groups could not be made of strictly equal size.

The groups were initially marked with a string and a plastic-laminated sign on a post. However, the beneficiaries soon removed the marking on preventive grounds, fearing that it would provide an entertainment for local children. The signs that were nevertheless left in place did, indeed, eventually suffer this fate.

Illustration 4: Tobacco experimental field in XiaoLongDong (小龙洞) township

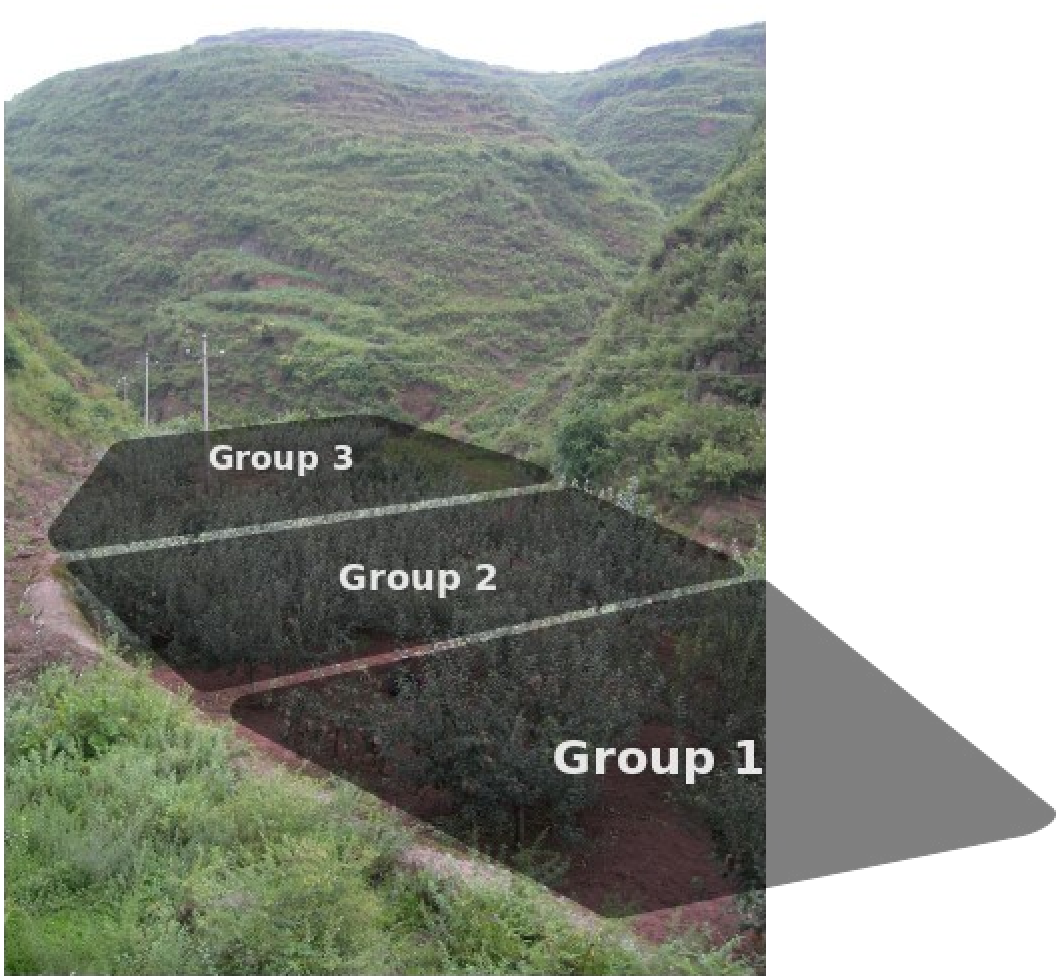


Illustration 5: One of the two orchards in Sayu (洒渔) township

3.1.6 Baseline monitoring

To ensure the accuracy of the follow-up afterwards, a baseline assessment of pests & diseases was carried out. In a first step, we conducted a survey of all the pests & diseases the farmers had observed in the last few years to narrow the range of all possible target pests & diseases on apple trees and tobacco. This was followed by terrain visits, and the samples obtained were taken to the laboratory of Yunnan Agriculture University. The species of insects and fungi were identified under microscope with the help of professors in the relevant fields. Some local names used by farmers had to be matched with common names and some were corrected.

Table 3 gives an outline of the pests and diseases identified on apple tree prior to the start of the experiment.

Name	**Incidence in previous years** Ben 1	**Incidence in previous years** Ben 2
Red Spider	Low	Low
Apple Leaf Defoliation Disease	Low	High
Root rot	High	High
Dry rot	High	High
Powdery mildew	Low	Low
Mosaic virus	High	High
Brown spot	Low	Low
Woolly aphid	Low	High

Table 3: Baseline pest&disease assessment - apple trees

3.1.7 Selection of the target pests and diseases

Although there are many kinds of pests & diseases affecting apple trees and tobacco, only those respecting the following criteria were selected as targets for quantitative monitoring:

- presence has been assessed on the selected field (according to baseline)
- homogeneous initial incidence on the whole plot, and in particular on the two/three subgroups
- potential effectiveness of bio-slurry (suspected, or according to previous knowledge/literature[1])

Apple

On apple trees, bio-slurry may be effective on Apple Leaf Defoliation Disease, root rot, dry rot, powdery mildew, mosaic virus, brown spot, woolly aphid and red spider[2]. However, owing to the limit of our instrument and technical means, we could not properly monitor soil-born diseases such as root rot and dry rot. Therefore, the experiment targeted the use of bio-slurry on leaves only. The initial morbidities of mosaic virus in the three groups were very different, so this disease was disqualified as a target pest. Brown spot and Apple Leaf Defoliation Disease were not selected because of very low morbidities in the orchards. So, finally, only powdery mildew, woolly aphid and red spider were selected as target pests & diseases.

Tobacco

On tobacco, bio-slurry may be effective against tobacco mosaic, brown spot, tobacco weather fleck, black shank and anthracnose, according to literature and internet sources[3,4]. Black shank and anthracnose were not selected as target diseases since there was only one plant in Group 2 infected by black shank and no anthracnose at all. Thus, tobacco mosaic, brown spot and tobacco weather fleck were finally selected as target diseases to follow up.

1 Zhang Jinliang. The Technology of Bio-slurry on Pests and Diseases. Inner Mongolia Agricultural Science And Technology, 2011.

2 Zhen Naixin. The Testing Experiment on Fruit Trees Prevented By Bio-slurry, 2006.

3 Zhang Wudi. The Effect Analysis of Bio-slurry on Curing Pests and Diseases. Research of Agricultural Modernization, 2001.

4 Tobacco Growers Information, North Carolina Cooperative Extension. http://tobacco.ces.ncsu.edu/

3.1.8 Sample size and sample selection

3.1.8.1 Apple

5 trees were chosen in each group (which contains typically 10 to 35 trees). As much as possible, the following criteria were followed for tree choice:

- mature tree, average to large-sized
- Fuji cultivar (some other cultivars were also found in the orchard)
- not immediately at the interface with another group, preferentially in the centre

The same trees were used for all successive investigations, and were therefore numbered and marked with a plastic-laminated tag noting the group number and tree number. Following identical trees over the season permits additional time-related verifications, at the expense of potential higher biases at the tree selection stage.

Illustration 6: Tagged tree

3.1.8.2 Tobacco

The investigation of diseases on tobacco was based on a five-point sampling method (four points in every corner of the square field and one point in the middle). According to the amount of tobacco plants (about 200 plants in each group), 20 samples in each group were considered adequate to guarantee the validity of the investigation. Two field screenings were done at three weeks interval, for which different plants were chosen (no tags were hung).

3.1.9 Frequency and duration of monitoring

Considering the frequency of spraying bio-slurry and pesticides, as well as the duration of the life cycle of most pests and diseases, we came to the terrain every two or three weeks, avoiding the rainy days because some pests or fungi might be washed away. We also tried to avoid the time just after farmers trimmed the infected branches since it would affect the accuracy of the results. However, the farmers trimmed irregularly, so we gave up taking this factor into consideration.

On apple trees, the monitoring started simultaneously with the first spraying at blooming, in March. The monitoring was terminated three weeks after the last spraying of bio-slurry, which took place in July. The spraying of bio-slurry should better be interrupted in advance to respect a safe retrieval time before harvest and avoid any contamination of the apples by pathogens in the bio-slurry (a similar consideration applies for pesticides).

3.1.10 Quantitative monitoring

Several approaches come to mind when designing a quantitative monitoring protocol. In most cases, the number of potential sites (leaves, branches, pruning scar...) on which to scan for the pest or disease are overwhelmingly large, therefore a sub-sample of the plant has to be selected on which to observe the presence of the disease. This sub-sample, typically a predetermined number of leaves, has to be representative for the tree and selected according to common rules on every plant (cardinal directions, height, etc...).

Two approaches to counting can be adopted. In the first one, a quantified value representing the prevalence is noted for every observation site. Either the pest or disease is directly quantifiable, such as the number of insects or fungal spots per leaf, or it has to be otherwise quantified, such as the percentage surface of leaf affected by a fungus.

In the second approach (“YES/NO” approach), the observation site is simply noted as positive or negative according to a determined criteria (ex: positive if at least one red spider is observed, or if at least some cottony residue from woolly aphid is spotted). This approach is best suitable for pests and diseases that are difficult to quantify. Besides, it requires less time and simpler means of observation.

The first approach therefore yields several observation values per plant (as many as observation sites), while the second approach will result in only one value per plant (typically, the ratio of positive observations). Statistical analysis can therefore be finer using the first approach (nested ANOVAs could be performed).

In this experiment, we used exclusively the second approach (YES/NO), because many pests were too small to count (ex: red spider), and most fungi were difficult to quantify (ex: powdery mildew on branch tips).

3.1.10.1 Apple

As explained above, the investigation was conducted on five selected/tagged apple trees in each group. On each apple tree, twenty leaves, branch tips or bark scars were chosen to observe the presence of woolly aphids, powdery mildew and red spider. These observations sites were taken equally on the four cardinal directions and different heights. The detailed protocol is presented in table 4.

In the preliminary phase, we only observed woolly aphids collecting on pruning scars. However, with the spreading of this pest later in the season, we also discovered them occurring on leaves. So we divided the observation of "woolly aphids" into "woolly aphids on scars" and "woolly aphids on leaves". It is essential to separate them into two parts to follow up in the absence of a microscope, since the woolly aphid is too tiny to be counted with naked eyes and the size of woolly aphids' gathering spots on leaves is sometimes bigger than that on scars, which means there are more woolly aphids on leaves.

Target pest or disease	Monitoring protocol
Woolly aphids on leaves	Observe 20 leaves Leaf is positive if at least 1 woolly aphid is spotted
Woolly aphids on scars	Observe 20 scars Leaf is positive if at least 1 woolly aphid or cottony residue is spotted
Powdery mildew	Count 20 branch tips Leaf is positive if any mildew is spotted
Red spider	Count 20 leaves Leaf is positive if at least 1 red spider is spotted

Table 4: Monitoring protocol on apple tree

3.1.10.2 Tobacco

As explained above, twenty tobacco plants were chosen every time by five-point sample method. On each plant, all leaves were observed for the presence of tobacco mosaic, brown spot, and tobacco weather fleck. The total amount of leaves and the number of leaves infected by each disease on every plant were noted.

Target pest or disease	Monitoring protocol
Tobacco mosaic	Observe all leaves Leaf is positive if the disease is spotted
Brown spot	Observe all leaves Leaf is positive if the disease is spotted
Tobacco weather fleck	Observe all leaves Leaf is positive if the disease is spotted

Table 5: Monitoring protocol on tobacco

4 RESULTS AND DISCUSSION

4.1 Bio-slurry characteristics

All the bio-slurry used for the field experiments was sourced from the beneficiaries' own biogas digester, which were 10m3 fixed-dome "Chinese model" digesters built with the help of ID China between 2009 and 2012.

For all the experiments described here, the bio-slurry was extracted from the water chamber, and was therefore generally free from solid particles. For use in the sprayer, the liquid was however filtered either through a piece of cloth or through the integrated sieve (illustration 7). No clogging of the spraying nozzle was reported.

Table 6 presents the typical chemical characteristic of bio-slurry as we assumed for this report. These figures are averages of 6 samples taken in April 2013 from three biogas digesters on ID project sites. On each digester a sample of liquid bioslurry was taken from the water chamber, and a sample of semi-solid bio-slurry was taken from the floating scum through the main lid. Note that these are not chemical analysis of the bio-slurry actually used for the experiments, however, so the figures should be taken as typical values only.

Illustration 7: Filling of the electrical sprayer with bio-slurry, diluted 1:1 with water

	pH	Humidity %	Organic Matter %	Total N %	Rapidly available N %	Total P_2O_5 %	Total K_20 %
Liquid fraction	7.6	99.01	0.49	0.12	0.10	0.02	0.04
Solid fraction	7.4	92.84	3.82	0.24	0.12	0.04	0.04

Table 6: Typical chemical analysis of bio-slurry

4.2 Apple

4.2.1 Treatments

4.2.1.1 Beneficiary Ben 1

● Spraying
× Field monitoring
▼ Harvest begins

The complete treatments used by Ben 1 on the three groups of his apple orchard are shown in table 7. Table 8 summarizes the pesticides that were used by the beneficiary on groups 2 and 3. This data is compiled from the logbook that the beneficiaries kept up to date after each application of pesticide or bio-slurry.

<table>
<tr><th>Group 1
bio-slurry only</th><th>Group 2
bio-slurry and pesticides</th><th>Group 3
pesticides only</th></tr>
<tr><td colspan="3">10 kg of lime-sulphur mixture (石硫合剂) in early March</td></tr>
<tr><td>30l of bioslurry diluted with 15l of water on the following dates:</td><td>10l of bioslurry diluted with 10l of water on the following dates:</td><td rowspan="2"></td></tr>
<tr><td colspan="2">2 April - 19 April -16 June -3 July</td></tr>
<tr><td></td><td colspan="2">Pesticides as described in table 7</td></tr>
</table>

Table 7: Summary of treatments applied on Ben 1's apple orchard

Table 8: Summary of pesticides used by Ben 1 on groups 2 and 3

Pesticide	Usual quantity (one 20l sprayer, = 30 trees)	Frequency of application Time period	Total number of applications
已唑醇 Tebuconazole	10g	once Beginning of April	1
毒死蜱 *Chlorpyrifos*	*10g*	3 weeks Begin March- end July	*5*
5%高效氯氟氰菊酯 Lambda-Cyhalothrin	10g	3 weeks Begin March- end July	*5*
代森锰锌 Mancozeb	30g	3 weeks Begin March- end July	*5*
多菌灵 *Carbendazim*	*30g*	3 weeks Mid March- end July	*4*
有机硅 Organosilicone surfactant	10ml	3 weeks Mid March- end July	*4*
甲基硫菌灵 Thiophonate-methyl	7g	3 weeks Mid March- end July	*4*
吡虫噻嗪酮 Imidacloprid buprofezin	10g	3 weeks Mid March- end July	*4*

4.2.1.2 Beneficiary Ben 2

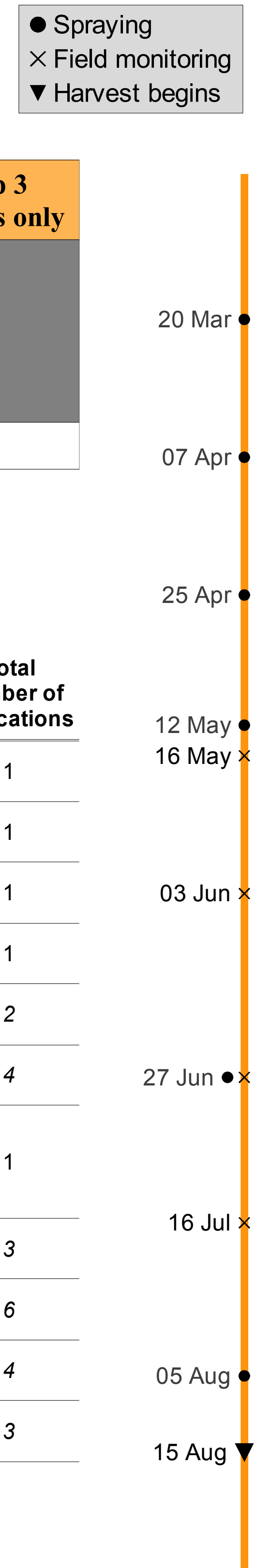
The treatments for Ben 2 are given in tables 9 and 10.

Group 1 bio-slurry only	Group 2 bio-slurry and pesticides	Group 3 pesticides only
25l of bioslurry diluted with 35l of water on the following dates:	25l of bioslurry diluted with 35l of water on the following dates:	
20 March - 7 April - 25 April -12 May - 27 June		
	Pesticides as described in table 9	

Table 9: Summary of treatments applied on Ben 2's apple orchard

Table 10: Summary of pesticides used by Ben 2 on groups 2 and 3

Pesticide	Usual quantity (for 2 20l sprayers = 15 trees)	Frequency of application Time period	Total number of applications
三唑酮 Triadimefon	45g	Once Mid-March	1
啶虫脒 Acetamiprid	10g	Once Mid-March	1
三唑酮 Triazolone	50g	Once Mid-April	1
毒死蜱 *Chlorpyrifos*	*30g*	Once Mid-April	1
5%高效氯氰菊酯 Beta-cypermethrin	50ml	Twice Beginning July, beginning August	*2*
代森锰锌 Mancozeb	36ml	Every 2 weeks End April – begin August	*4*
氟硅.戊唑.乙 *25% flusilazole* *12.5% tebuconazole* *25% ethylicin*	*75ml*	Once End April	1
有机硅 Organosilicone surfactant	20ml	Every 2 weeks End April – begin August	*3*
甲基硫菌灵 Thiophonate-methyl	50g	Every 2 weeks Mid-March – begin August	*6*
阿维甲氰 Abamectin	50ml	Every 2 weeks End-April – begin August	*4*
多菌灵 Carbendazim	20ml	Every 2 weeks Mid-June – begin August	*3*



4.2.2 Pest and disease monitoring

Four field visits were undertaken for the quantitative counting of pests and diseases on the orchards.

4.2.2.1 *Woolly aphids on scars*

The woolly aphids on apple trees were both monitored on scars and on leaves. The size of their gathering spots on leaves are usually larger than those on scars.

Illustration 8: An old pruning scar showing typical signs of a colony of woolly aphids

The results of the quantitative monitoring are shown in illustration 9 for the two orchards. In early June, woolly aphids arrived at occurrence peak in both Ben 2 and Ben 1's orchards. As it can be seen, the differences between the 3 groups are usually within the standard error. The significance of the results was analysed using a one-way ANOVA for each field visit (thus eight separate ANOVA in total, four on each orchard). Within each of these ANOVA, the results were compared one by one using a Tukey-Cramer test at a significance level of 0.05. Out of the 24 combinations (2 beneficiaries times 4 ANOVA times 3 comparisons amongst groups), only one was found to be significant (Ben 2: group 1 versus group 2 on the 2013.6.3).

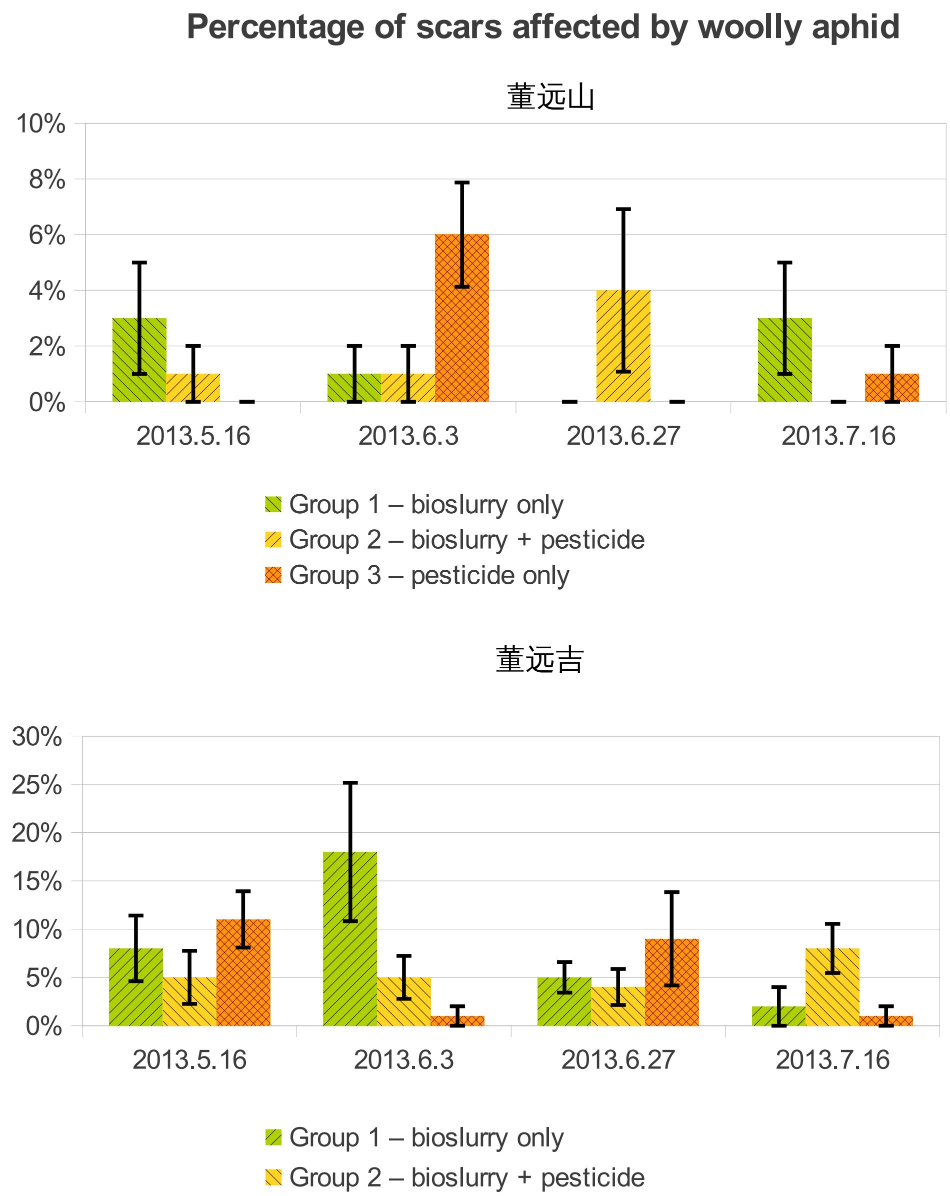


Illustration 9: Incidence of woolly aphid on bark scars. Standard error is shown. When no bar is present, value is zero.

Furthermore, as can be seen the ranking of the different groups was not consistent from one field visit to another. No pattern can emerge placing incidence rates of one group systematically, or at least predominantly, above another.

In the light of the inconsistency of these results, it is assumed that the statistical power of our test was not sufficient. Considering the very heterogeneous nature of the experimental orchards, the sample size ought to have been larger (more trees), or the count more precise (more leaves counted on each tree).

The heterogeneity in the orchard regarding the woolly aphid incidence is thought to be mainly due to the chronic recurrent nature of the pest. Indeed, woolly aphids do overwinter within the tree, in the roots or under loose bark, so that morbidities on a specific tree are heavily influenced by the previous year's populations. Previous incidence rates can be a factor more potent than the actual treatment applied. Therefore, an experiment continued on several years could be far more reliable for assessing the efficiency of a treatment with bio-slurry. The variation in incidence due to the recurrence of the pest also pleads in favour of a larger experimental sample.

In addition, the interaction by the farmer may have played an important, if not major, role. Indeed, the farmers used several times through the season a black tar-like product to cover the aphid-infected bark scars. In the context of such an experiment, we ought to have convinced the farmers from abstaining of using this method, but we failed to realise the issue early enough and to give instructions accordingly. To address the issue, we asked the farmers to treat all groups at once with the tar product, on the same day. However, this measure was not sufficient to guarantee the reliability of quantitative insect counts, which would require to completely suppress the smearing of tar.

In the light of these interactions between the experiment and the farmer's management, a better estimation of the efficiency of the different treatments may rely on the farmer's reported experience. Farmers were regularly asked about their perceived efficiency of the treatments.

Therefore, we asked an estimation of when and how much of the tar-like product had to be painted on each group. However, the farmers could not give an objective estimation of the latter, because they did not keep record, nor followed a regular routine.

Concerning the efficiency, Ben 2 reported that in his opinion the bio-slurry did not seem very effective against the woolly aphids. Ben 1 considered it useful to some extent, and said he would continue using it in the following years.

4.2.2.2 Woolly aphids on leaves

On the leaves, the amount of woolly aphids recorded was only sufficient for measuring statistics on one orchard (illustration 10). On the other orchard, the incidence was too low (absent or below 2%) on all 3 groups, so this data has not been retained in the study. Data was only obtained from three field visits, since the monitoring on leaves was started later.

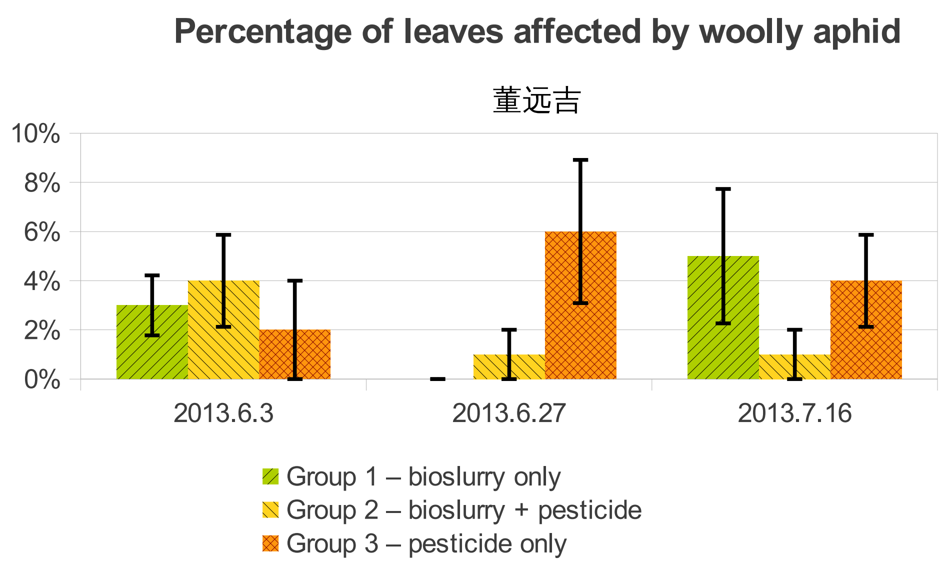


Illustration 10: Incidence of woolly aphid on leaves. Standard error is shown. When no bar is present, value is zero.

As can be seen from illustration 10, the results are not more statistically significant than on the pruning scars, and furthermore present inconsistent ranking between the groups through time. Most remarks mentioned above concerning the counting of woolly aphid on the bark scars are therefore also valid here.

4.2.2.3 Powdery mildew

Illustration 11: The symptom of powdery mildew on leaves

In our experimental orchards, powdery mildew was mostly noticed on the young branch tips, and therefore the fungus was only monitored there. No powdery mildew was noticed on the fruits.

The results of the quantitative monitoring are shown in illustration 7 for the two orchards. As it can be seen, the differences between the 3 groups are usually within the standard error. The significance of the results was analysed using a one-way ANOVA for each field visit (thus eight separate ANOVA in total, four on each orchard). Within each of these ANOVAs, the results were compared one by one using a Tukey-Cramer test at a significance level of 0.05. Out of the 24 combinations (2 beneficiaries times 4 ANOVA times 3 comparisons amongst groups), only one was found to be significant (Ben 1: group 2 versus group 3 on the 2013.6.3).

Furthermore, as can be seen, the ranking of the different groups was not consistent from one field visit to another.

The remarks mentioned above concerning the quantitative monitoring of woolly aphid on bark scars are valid here also to explain the lack of consistency and significance of the results.

Indeed, powdery mildew overwinters in the form of ascocarps in shoots and buds, and is therefore also a chronic recurrent problem. High disease levels at the end of a season may increase the percentage of infected buds in the following year. Thus the lack of consistent results for powdery mildew also pleads in favour of a study continued on several years.

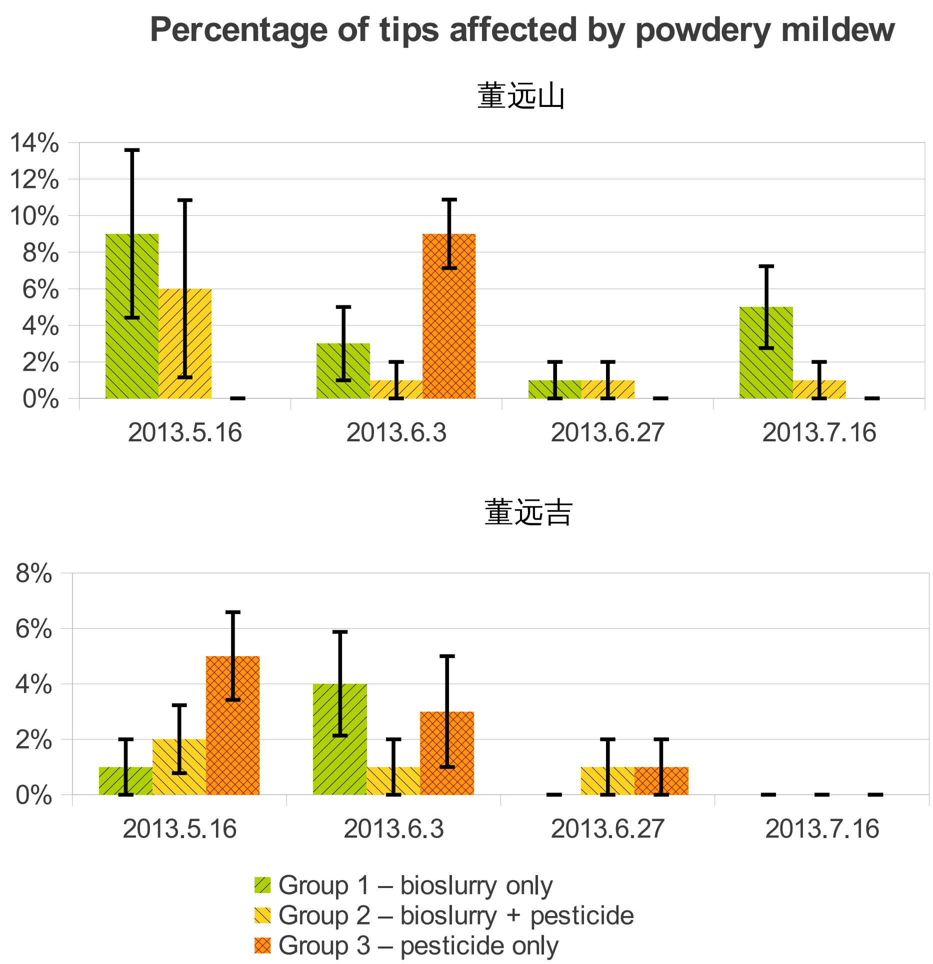


Illustration 12: Incidence of powdery mildew on branch tips. Standard error is shown. When no bar is present, value is zero.

Similarly to the painting of tar-like product on the bark scars, the farmers did regularly prune the branch tips affected by powdery mildew. Since this pruning happened at no regular schedule and was difficult to monitor, the quantitative results might have been largely affected. For potential future experiments, one might consider giving up altogether on pruning the infected tips, although this would suppress one of the most effective control measures against the fungus, and put the health of the trees at risk.

4.2.2.4 Red spider

The monitoring of red spider was meaningless in the two beneficiaries' orchards since there was almost no red spider, or they were too small to be observed. Therefore, the data on red spiders was not included in the results of this study.

4.3 Tobacco

4.3.1 Treatments

The two groups on the field received the following treatments (tables 11 and 12). As can be seen, group 1 received some pesticides in the early stage. Bio-slurry was only used twice on group 1, once in soil application and once in foliar spray.

Group 1		
Date	**Details of pesticide or bio-slurry**	**Quantity (whole group)**
2013.05.27	5公斤水+以下农药 5 kg water	
	代森锰锌 Mancozeb	15 g
	高效氯氰菊酯 Cyfluthrin	6 ml
	吡虫啉 Imidacloprid	5 g
2013.06.29	9公斤沼液+9公斤水泼叶面 9kg bio-slurry + 9kg water	9 kg

Group 2		
Date	**Details of pesticide**	**Quantity (whole group)**
2013.05.27	15公斤水+以下农药 15 kg water	
	代森锰锌 Mancozeb	*45g*
	高效氯氰菊酯 Cyfluthrin	*15ml*
	吡虫啉 Imidacloprid	15g
2013.06.28	20公斤水+以下农药 20 kg water	
	代森锰锌 Mancozeb	*45g*
	高效氯氰菊酯 Cyfluthrin	*15ml*
	吡虫啉 Imidacloprid	15g

Table 11: Pest control treatments applied on the tobacco field (foliar)

	What	**Quantity kg/mu**	**When**
Groups 1 & 2	Cow manure	600	29th of April
	13-11-24	85	29th of April
	Organic matter & micro-nutrients	50	29th of April
Extra group 1 only	Bio-slurry (soil application, poured at the foot of each plant)	400	10th of May

Table 12: Fertilisers applied on the soil

Owing to the fact that the experiment on tobacco was not initially planned, we missed the best monitoring period and the only two monitoring missions were not enough to ensure the representativeness of the final result. In particular, no monitoring was made prior to the first application of bio-slurry to assess the initial incidence of diseases.

4.3.2 Pest and disease monitoring

Two field visits were carried out for pest and disease quantitative monitoring on the tobacco. The results are shown in illustration 14. The three target diseases were tobacco mosaic, brown spot and tobacco weather fleck. The monitoring of powdery mildew was also planned; however, there did not exist any powdery mildew. Perhaps the fungus had not spread to this field. Or perhaps bio-slurry had good efficiency on powdery mildew in Group 1 and the pesticides controlled the disease in Group 2.

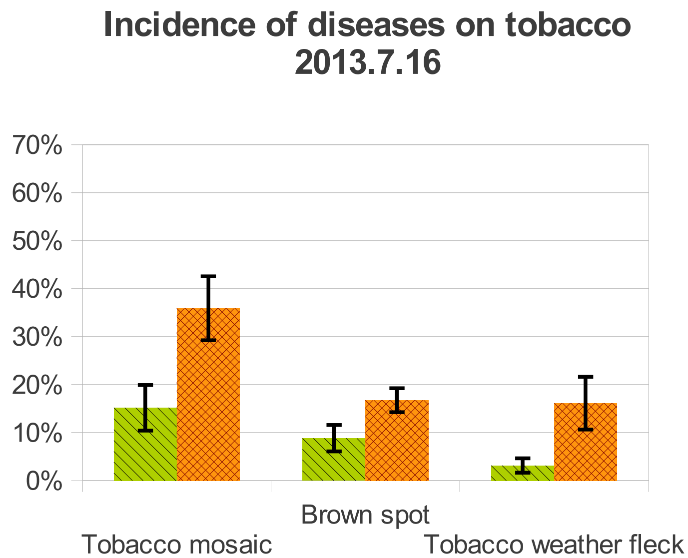
The significance of the results was analysed with a student's t-test for each pest and each field visit. Results are shown in table 13. At a level of significance of 0.05, 5 results out of 6 are significant.

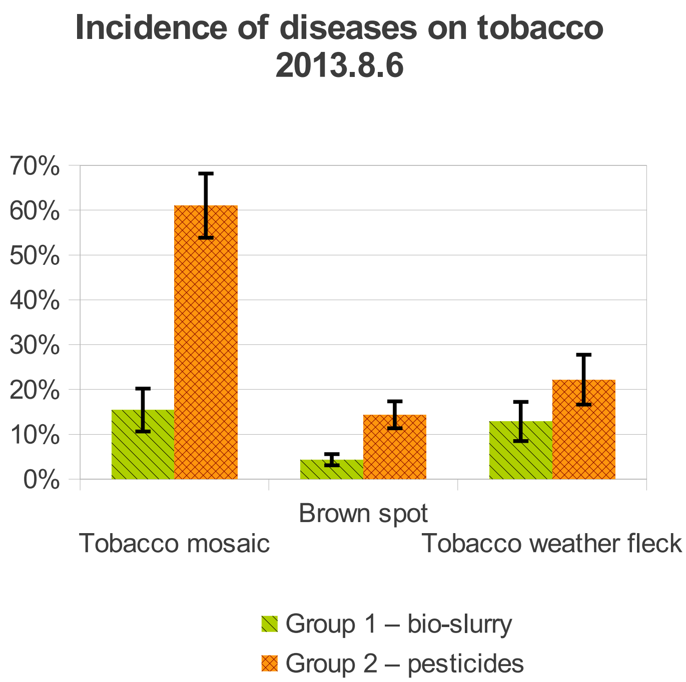
Illustration 13: Brown spot on tobacco

Table 13: Student's t-test of significance for tobacco pest and diseases

		2013.7.16			2013.8.6		
		Average	**SE**	**p**	**Average**	**SE**	**p**
Tobacco mosaic	**Group 1**	15.2%	4.7%	0.016	15.4%	4.8%	5.48E-06
	Group 2	35.9%	6.7%		61.0%	7.2%	
Brown spot	**Group 1**	8.8%	2.7%	0.039	4.3%	1.2%	0.004
	Group 2	16.7%	2.5%		14.3%	3.0%	
Tobacco weather fleck	**Group 1**	3.1%	1.5%	0.028	12.9%	4.4%	0.197
	Group 2	16.1%	5.5%		22.2%	5.6%	

Furthermore, and contrary to the results on the apple trees, the ranking of the different groups is consistent within the two field visits. For all three diseases, the incidence was systematically lower on the bio-slurry treated group.





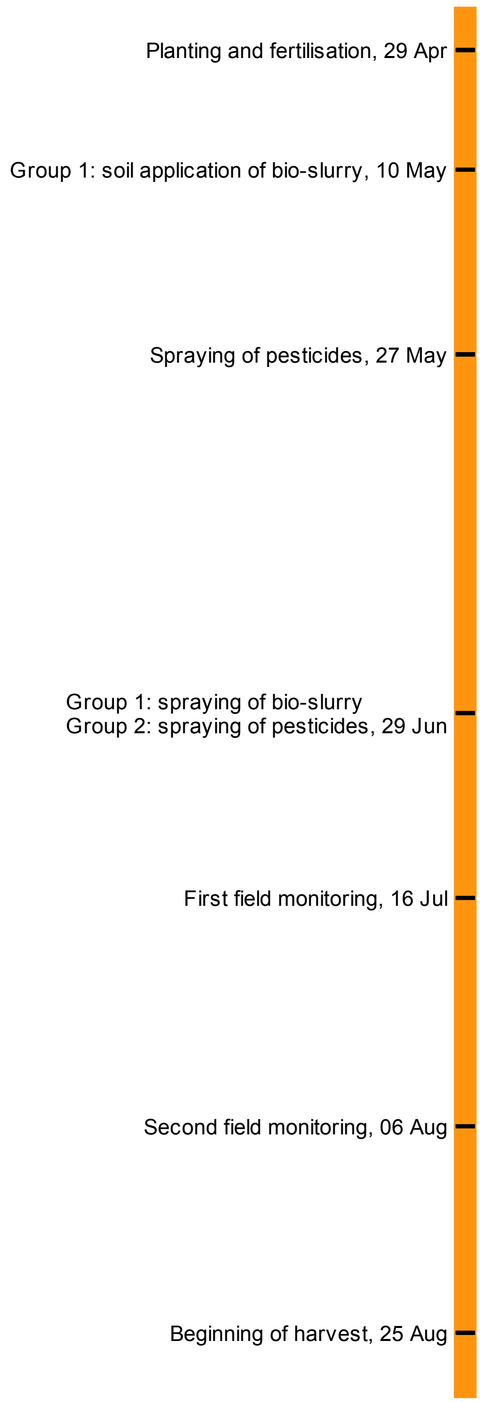


Illustration 14: Incidence of diseases on tobacco. Standard error is shown.

4.3.3 Conclusions

In the light of these results, it can be assumed that the treatment of the tobacco with bio-slurry was effective against the three target diseases of this study (with a good level of confidence for tobacco mosaic and brown spot, medium confidence for tobacco weather fleck). As said above, in the absence of a control group, it cannot be said whether bio-slurry also controlled powdery mildew, or whether the fungus did not enter the field in the first place. In tobacco mosaic and brown fleck, the stability (or even reduction) of the incidence on the bio-slurry group from the first visit (16 July) to the second (6 August), while the morbidity on the other group kept on increasing, could possibly indicate a preventive action of the bio-slurry. The experiment was limited in scope and no preliminary monitoring of the field was done before the first use of bio-slurry to exclude initial discrepancies, so the results should be taken with some care.

At this stage it is difficult to assess whether the efficiency of the bio-slurry was due to the direct effect on the pests, or perhaps to an indirect effect through increased fertilisation, or both. Indeed, not only was bio-slurry used on the soil on group 1, it potentially also contributed to the fertilisation though foliar absorption. The colour of the leaves was indeed distinctly darker green on group 1 (bio-slurry) compared to group 2. Plant higher in nutrients could perhaps have better withstood the diseases.

Furthermore, the reduction in disease incidence, particularly mosaic virus, could perhaps be linked to the control of its vectors. Tobacco mosaic virus is well known to be transmitted from one plant to another by insect bites, such as aphids or leafhoppers. Since the potential vectors were not monitored, no conclusion can be reached.

Concerning the fertilisation, a general advise in the cultivation of tobacco is that the supply of nitrogen should be restrained in late season to allow for proper leaf maturing. Leaves should turn yellow on the plant before harvest and subsequent curing. For this reason, the farmer decided to stop the spraying of bio-slurry after only one application, as soon as the effects of increased fertility became apparent. Consequently, we did not carry the pest screening after the first week of August.

The leaves from group 1 did indeed face some curing problems and achieved a lesser grade at selling point (for more details, see the report “Using bio-slurry for fertilisation”). If it is proved that the increased fertility was due (at least partly) to foliar absorption, this drawback could potentially be an obstacle to the spraying of bio-slurry on tobacco for pest control, unless the quantity of soil-applied fertiliser is diminished accordingly.

From the farmer's point of view, the efficiency of the bio-slurry on both the fertility and the control of the diseases was tangible. The farmer has announced his intention to experiment further with bio-slurry in the following year, but with a reduction in mineral fertilisers.

On tobacco, pesticides could perhaps be completely replaced by bio-slurry not only because of its specific fungicide effect, but also because it is reported to expel some pests such as plant hopper and aphid. However, it would be necessary to spray pesticides on leaves once in early growing period of tobacco if the beneficiaries doubt the efficiency of bio-slurry as pesticides.

4.3.4 Comparison with the apple orchard trial

By contrast with the results on the apple orchards, the significance of the results on tobacco were presumably favoured by the following factors:

- the homogeneity of the field was excellent, since
  - tobacco is an annual plant, grown from seed in the farmer's own nursery, and therefore is not differentially affected by chronic recurrent diseases from previous years
  - the crop is planted in regular tight rows and is therefore little affected by differential environmental conditions within the field
  - the number and density of tobacco plants form a quasi-continuum, while trees in the apple orchard were distinctively singular
  - the environmental conditions on the two parts of the field (groups 1 and 2) were very similar (soil, slope, sun, water)
- the larger size of the sample: 20 tobacco plants were screened in each group (versus 5 trees per group in the apple orchards)
- since every tobacco plant was screened entirely (every single leave of the plant was screened for disease), biases of representativeness have been avoided. However, the size of the sample was lower than on apple trees: 12 to 15 tobacco leaves per plant, compared to a sub-sample of 20 leaves per tree.

Illustration 15: Tobacco mosaic

4.4 Influence of the weather

Our results have been influenced by the weather. If it rained heavily before the investigation time, some pests might hide and the fungi might be washed away.

Most of our observations seemed indeed to have been largely weather-dependant. For best results, similar weather conditions should be chosen for each monitoring mission. The weather should be favourable to the observation of the pests. Monitoring during the rain or in the days following rain should be avoided.

4.5 Possible damages from bio-slurry

Some “burnt” spots, resembling the symptoms from excessive pesticide usage, were discovered on the leaves of all three groups of apple trees, with more incidence in the two groups on which bio-slurry was sprayed. Furthermore, another farmer reported that the apple leaves curled seriously after applying undiluted bio-slurry.

According to the beneficiaries, it is possible that excessive amounts of bio-slurry, or under-dilution with water, could be the cause for the burnt spots. It is believed that increasing the dilution ratio could reduce or suppress the problem, but at the likely expense of the slurry potential effectiveness against pests and diseases.

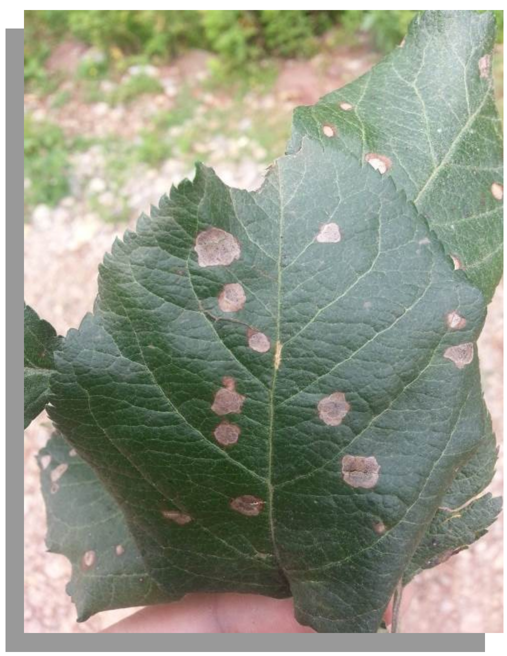


Illustration 16: Burnt sports on apple leaf

RECOMMENDATIONS FOR FUTURE EXPERIMENTATIONS

- The experiments should be prolonged for several years especially if investigating recurrent chronic diseases
- For homogeneity reasons, the plots should be on flat ground and cropped similarly in a regular arrangement. The plots should be screened beforehand for baseline discrepancies in pest/disease incidence.
- The selected fields should have high relative pests and diseases incidence in previous years.
- A control group (no treatment) should be established
- For apples, we would advise that every group should contains at least 35 trees, of which 10 or more are investigated at each visit. For tobacco, 200 plants per group on which 20 are investigated seems sufficient.
- On apples, we would advise that each tree is screened in at least 40 spots (whether leaves/branch tips/pruning scars...) in all cardinal directions and heights. On tobacco, all leaves should be screened. Noting an incidence rate for each spot (rather than mere presence/absence of disease) would enhance the quality of the data.
- Intervals between field visits should ideally be 2 weeks for apple, and could even be reduced to 1 week for tobacco if the bio-slurry spraying period is short.
- Clear instructions have to be given to the farmers, pruning and painting tar should be avoided, or at least done at known times.
- Field visits should not be done in the rain or in the few days following heavy rain. If the weather prediction is inaccurate, it is safe to plan a longer stay on the terrain.

5 CONCLUSIONS

The part of the experiment on tobacco showed obvious differences between the two treatments, supported by statistically significant results. Although bio-slurry was only applied once on the soil and once in foliar spray, there is therefore some evidence to indicate that it was effective on the reduction of three diseases. For various reasons indicated above, the results should be taken with care. Furthermore, bio-slurry may also have negative side-effects on curing. The beneficiary has been convinced by the effectiveness of bio-slurry and is planning to carry on experimenting with bio-slurry in the following years, hopefully encouraging the use in his village.

The other part of the experiment on apple trees gave mixed and uncertain results. Although the beneficiaries reportedly noticed some bio-slurry efficiency, the results were overall not tangible, and statistical analysis showed no significant differences between the treatments. For a better accuracy, the conditions within the orchards ought to have been more homogeneous, and/or the statistical power increased. Undoubtedly however, bio-slurry did not bring a radical control of the pests.

Nevertheless, on the question of the relevance of launching a large-scale project for developing the use of bio-slurry as pesticide amongst ID beneficiaries, the lack of tangibility and observability in the apple experiment is in itself a worthy outcome. Without obvious observable benefits for the people, a potential rural development project stands no chances of success. Regardless of whether the lack of results were due to the experiment inaccuracies or to the effect of bio-slurry being too limited, designing an overly sophisticated experiment is not necessary: if the efficiency of bio-slurry was large enough to constitute a solid motivation for the beneficiaries, then a basic experiment of limited statistical accuracy should be sufficient for confirmation.

For a small organisation such as ID China (with no pretensions to scientific research whatsoever, besides the necessary knowledge for improving the quality of our development programs), state-of-the-art agronomic research is out of reach. On the other hand, small-scale applied research might be too unreliable. In particular, the challenges of bringing a team of town-based agronomists to work together with small-scale farmers on a terrain-oriented research have become clear. Far from the research institute, field trials face many external and internal interactions. Although the cooperation with the farmers was globally good, many aspects of the experiments were not under control. Throughout the project, communication remained an issue, and many times the need for objective rigour collided head-on with the rural and human realities.

Therefore, perhaps another lead to explore for assessing the efficiency of a given technical opportunity would reside in community testing rather than in a few in-depth experiments. For example, a larger group of farmers could be supported in experimenting the use of bio-slurry, with a much looser involvement from ID China. After some time of testing, the solutions providing a tangible benefit would get naturally selected and with some support, could be adopted by the farming community.